Final Report 2-11-04

The Current Status of HIV-1 Vaccine Development, 2004: Recommendations for the Future

By

The NIAID AIDS Vaccine Research Working Group*

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Potential Conflict of Interest Statement

Many of the AVWRG members are actively working on aspects of HIV-1 vaccine development. Hence, committee members acknowledge that they can have conflicts of interest, to varying degrees, with some of the priorities and recommendations noted in this report.

Executive Summary AIDS Vaccine Research Working Group Report

Since the first AIDS cases were reported in the US in 1981, more than 65 million people world-wide have been infected with HIV-1. To control the global HIV-1 pandemic, development of a safe, practical and effective vaccine is urgently needed. The problems that continue to face HIV vaccine development are the:

1. lack of an immunogen that will induce antibodies that broadly neutralize HIV primary isolates,
2. lack of an immunogen that will induce effective T cell responses against diverse HIV isolates,
3. lack of an immunogen, adjuvant, carrier or delivery strategy that will enhance the duration of immune responses enough to make a vaccine practically useful over time,
4. lack of an immunogen that induces protective immune responses with one or two primes/boosts,
5. lack of an immunogen or strategy to induce protective innate immunity, and
6. lack of an immunogen that is accessible and affordable world-wide.

This report reviews selected aspects of the current status of HIV vaccine research, and makes recommendations for improving the development and testing of promising HIV experimental vaccines.

Summary of AVRWG Report Recommendations

Design of Strategies to Overcome HIV-1 Diversity

1. Formalize the development of a database of newly transmitted HIV-1 full-length sequences for construction of subtype and group centralized sequences for antigenicity and immunogenicity studies. Include the sequences of breakthrough isolates from all HIV-1 vaccine trials.

2. Make the sera available from all clinical trials so that comparative studies can be performed between sera generated against all newer immunogens to determine if any induced antisera have more breadth than that generated by immunizing with AidsVax immunogens.

Development of Immunogens and Animal Models to Optimize Induction of Durable Anti-HIV-1 T and B Cell Responses

1. Accelerate basic science studies into new live vector development to better understand the issues of pre-existing immunity to current vectors being developed, and induce durable immune responses. For example, proof of concept studies should be performed quickly in order to understand the role of preexisting immunity on utility of recombinant adenoviral vectors, Modified Vaccinia Ankara (MVA) and other vectors in the pipeline. Vectors should be specifically sought for which pre-existing immunity is not an issue, and that

induce long-lived immunity with one or two immunizations, with acceptable safety profiles.

2. Focus basic research on new immunization strategies for enhancing induction of memory T and B cell pool size, including determining the role of modulating T regulatory activity on the efficacy of HIV-1 immunization strategies.

3. Develop overlapping peptide pools for use in comparative immunogenicity studies of HIV-1 vaccine candidates.

4. Accelerate adjuvant development, and synergize with the new efforts of NIAID with emerging infections and biodefense research on Toll-like receptor ligands, cytokines, and other stimulatory molecules of dendritic cells, B and T cells as targets for new adjuvants.

5. Development of adoptive transfer models to help define preclinical and clinical advances in immunogenicity.

6. Define how we can better correlate animal immunogenicity of HIV-1 immunogens with human immunogenicity.

7. Develop new animal challenge models to determine protective anti-HIV-1 T and B cell responses. These models should include low dose mucosal SHIV (to test Ab-based vaccines) and SIV challenge models.

8. Test mixtures of vectors for optimizing HIV-1 vaccine immunogenicity..

9. Consider a new effort to define the correlates of protection for *nef*-deleted attenuated SIV, as these correlates are still ill-defined. Define the role for other HIV regulatory genes as vaccine components.

10. Define the mechanism of anti-host cell antibodies in protection from SIV challenge.

11. Define correlates of protection to HIV in other animal models of SIV/SHIV infection and in human clinical trials.

12. Define the comparative roles of free virus and cell-associated virus in the pathogenesis of HIV-1 infection, ie for design of effective vaccines, we need to know more about transmission modes at mucosal and systemic sites.

13. Encourage development of a "Human Challenge Model" of HIV-1, wherein HIV-1+ patients on HAART would be vaccinated with the more promising experimental immunogens. If they then elect to stop receiving therapy, the ability of the vaccine-induced immune responses to prevent, delay or modify the subsequent increase in plasma viremia could be a useful way to gauge vaccine

potency. However, it must be noted that this experimental system is likely to be a more stringent test of the potency of a vaccine, because the amount of replicating virus to be countered is greater than is involved in de novo infection, and because of the possibility that the pre-existing HIV-1 infection has already caused a significant level of immune impairment in the volunteers, despite their receipt of therapy. A mechanism would need to be found to provide the drugs for such a trial.

Strategies For Design of Immunogens to Induce Antibodies that Broadly Neutralize HIV-1 Primary Isolates

1. Increase funding of basic research to develop novel approaches for immunogen development for neutralizing antibody induction. Ensure continued and robust funding of Innovations grants programs, HIV-RAD and IPCAVD programs. Define how we rank order the need for basic research, and evaluate funded programs.

2. A bridge program or process is needed for successful R21 AIDS Vaccine Innovation grants to move their products forward, such as an R33 program.

3. Develop a database of newly transmitted HIV-1 isolates (see #1 above under strategies to overcome HIV-1 diversity).

4. Develop a standard panel of isolates to compare immunogens in an organized way to rank-order their potency for induction of neutralizing antibodies. Small but real improvements can be significant, pointing the way to viable strategies and possible combinations of strategies that would lead to greater potency. (This is already being planned by John Mascola and David Montefiori). It should be noted that a workshop aimed at gathering suggestions for a standard panel of isolates sponsored by the DAIDS, AVRWG was held on Jan. 6, 2004. Another goal of the workshop was to discuss a level of cross-neutralizing activity that warrants advancement to phase II and III trials.

Clinical Trials and International Research Issues

1. Establish and further define the process and criteria for moving candidate HIV-1 vaccines into Phase I, II and III clinical trials, to include vaccine candidate cost and manufacturability.

2. Coordinate the myriad of trials organizations, and continue the organizational efforts of PAVE by DAIDS with HVTN, CDC, DoD and other relevant parties, e.g. IAVI.

3. For preclinical and clinical development of vaccine products, develop the expertise for SOP development for data management, clinical trial end-point assay validation, and clinical trial site monitoring, and make these SOPs and expertise available to the vaccine development community.

4. DIADS should continue to pursue mechanism of research and development that do not rely on traditional peer-reviewed mechanisms, to be able to quickly fill research gaps and move products forward.

5. DAIDS should begin to target the HIV Team Contracts, HIV-RAD, IPCAVD, and R21 grants to problems that are needed to be addressed and away from concepts that are already being addressed in the NIH research portfolio.

6. Establish a centralized effort to compare serum and cellular reactivity induced by HIV immunogens prior to entry of immunogens into clinical trials. The important point is that all critical immune assays be unified under central standard operating procedures and possibly at a central laboratory in order for immunogenicity data to be compared. For example, continued support for the NIAID/DOD GLP QC and Immunogen Comparison Laboratory effort and consider expansion of capacity, in order for a central site for immunogen testing to be developed.

Community Issues

1. Actively promote the recruitment of women and people of color into future HIV-1 vaccine efficacy trials.

2. Develop practical solutions to barriers of HIV-1 vaccine availability and potential for manufacturing capability, in particular for developing countries.

3. Build adequate community information and education dissemination in the plans for continued trial network development, to ensure that expectations of the community are realistic.

VII. Summary

The relentless progression of the HIV-1 epidemic world-wide in spite of the world's efforts, emphasizes more than ever the need to accelerate, intensify and coordinate the process of HIV vaccine development. The field of HIV vaccine research has labored for nearly 20 years in the quest for an AIDS vaccine. Much has been learned and although some progress toward development of a safe, effective and practical vaccine has been made, major problems remain to be overcome. It has become apparent that if, indeed, it is possible to make an effective HIV-1 vaccine, then the successful and timely development of an effective vaccine will require focusing existing resources on clinical trials of the most promising candidates, coupled with concerted efforts in basic research to understand the correlates of protective immunity, and to develop novel solutions to neutralizing antibody immunogen design, vector design, and T cell immunogen design to overcome HIV-1 diversity. By synergizing basic research and clinical research efforts and focusing on the critical proof of concept

experiments, an iterative process can continue to move forward to bring promising experimental HIV immunogens into Phase III trials.

Full AIDS Vaccine Research Working Group Report

I. Introduction

Since the first AIDS cases were reported in the US in 1981, more than 65 million people world-wide have been infected with HIV-1. To control the global HIV-1 pandemic, development of a safe, practical and effective vaccine is urgently needed. The problems that continue to face HIV vaccine development are the:

1. lack of an immunogen that will induce antibodies that broadly neutralize HIV-1 primary isolates,
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6. lack of an immunogen that is accessible and affordable world-wide.

The primary advisory group to the NIAID for AIDS vaccine research scientific issues is the AIDS Vaccine Research Working Group (AVRWG), that is charged with advising NIAID and NIH on AIDS vaccine development research opportunities and directions. This report is a summary of new information presented at the recent international AIDS vaccine research conference, AIDS Vaccine 2003 held in New York September 18-21, 2003. In addition, members of the Working Group have incorporated additional information that has become known since the meeting to the report in order to present a current appraisal of the challenges and opportunities in the field. Most importantly, recommendations are presented to speed the design and testing of the most promising HIV vaccine candidates.

II. Vaccine Strategies to Contend with HIV Diversity

Overview of Immunogen Design to Overcome Genetic Diversity. Genetic variation of HIV-1 is a major obstacle for AIDS vaccine development. Since HIV-1 group M began its expansion in humans approximately 70 years ago, it has diversified rapidly, and now comprises a number of different subtypes and circulating recombinant forms (CRFs) (1-3). The HIV-1 group M is the set of diverse viruses that dominates the global AIDS epidemic. Subtypes are genetically defined lineages that can be resolved through phylogenetic analysis of the HIV-1 group as well defined clades or branches in a tree. Recombination occurs frequently, and a circulating recombinant form (CRF) carries sections of two or more HIV-1 subtypes in a mosaic genome; a recombinant lineage is designated a CRF with related forms found in multiple epidemiologically unlinked individuals, and two of these, CRF01 and CRF02, are dominant epidemic strains frequently found in Asia and Africa, respectively. Currently, strains belonging to the

same subtype can differ by up to 20% in their envelope proteins, and between subtypes, distances can soar to 35%. Moreover, this diversity is continually growing. The need for changes in the annual influenza vaccine puts into perspective the implications of such diversity—less than 2% amino acid change can cause a failure in the cross-reactivity of the polyclonal response to the influenza vaccine, and necessitates changing the vaccine strain (3).

The current scale of the HIV-1 pandemic world-wide makes action imperative to design vaccine strategies to contend with the extraordinary diversity of HIV-1. Over the years, the Los Alamos National Laboratory has created an extensive global database of over 80,000 HIV-1 sequences linked to geographic and subtype information. The database has also compiled an immunologic database of the thousands of HIV-1 epitopes recognized by T cells in the context of MHC Class I or II molecules, or recognized by antibodies (www.hiv.lanl.gov) (4,5). These databases provide a framework for a reasoned selection of vaccine candidates for testing.

The Centralized HIV-1 Immunogen Design Approach. In 2000, Korber and colleagues performed a computer analysis of HIV-1 sequences in order to time the ancestor of the HIV-1 pandemic strains, and estimated that the first index case, or most recent common ancestor of group M HIV-1 strains occurred in Africa in approximately 1930 (2). In this analysis, Korber constructed a parsimonious candidate for the ancestral sequence, which was a group M consensus sequence (2). From this work, and from additional work that had shown that known CTL epitopes were more persistently preserved in subtype and M group consensus sequences, came the concept of centralized HIV-1 immunogens (4).

To overcome the challenge of HIV-1 diversity, “centralized” HIV-1 genes in HIV immunogen design have been proposed (6-10). These strategies include using consensus, the most frequent base found in a given position, or ancestral or center of the tree sequences, both modeled from phylogenetic trees. Three computer models (consensus, ancestor, and center of the tree [COT]) have been proposed to generate centralized HIV-1 genes (6-10). The biology of HIV gives rise to a star-like phylogeny, and as a consequence of this, the three kinds of sequences are very similar to each other. Any of the three will reduce the protein distances between immunogens and field virus strains. In the case of the group M central sequences studied here, distances to all subtypes and recombinants are essentially reduced to intra-subtype levels (2,4,11,12). Within subtypes, distances are roughly halved. But given the fact that HIV-1 is diversifying under host immune pressure, the small number of differences between the three model sequences may be enriched for immunologically important positions. Consensus, ancestral and COT sequences, despite their similarities, have theoretical advantages and disadvantages (1,6,7,10). Global sequencing is generally conducted using viruses sampled during chronic infections that have been subjected to within-host immune pressure, not on transmitted virus sampled during acute infection. While consensus sequences are arguably the most representative of current circulating viral populations, ancestral and COT sequences hypothetically may have an advantage of re-creating potent epitopes that have tended to escape over time during chronic infections, but for reasons of viral fitness and transmission, tend to revert to a more ancestral form in a new host. But even phylogenetic reconstructions of COT and

ancestor sequences may miss such epitopes if they are inadequately represented in the sequences sampled and used to reconstruct the trees. Furthermore, focusing a vaccine response on epitopes that for the most part have escaped and are rare in a contemporary population may be a disadvantage, no matter how potent the response to a particular epitope.

Another potentially useful strategy would be to derive central sequences using only samples obtained during acute infection, but at this time such samples are inadequately represented in the database. Currently, there is some evidence that subtype isolates derived from early infection may be more susceptible to neutralizing antibodies (13). Although computer analysis has demonstrated that centralized HIV-1 sequences clearly decrease the genetic distances between centralized gene and wild-type sequences compared to distances among wild type sequences (1, 2), it is not known if centralized sequences could induce anti-HIV-1 responses to any wild-type T or B cell epitopes**.** Data presented at the AIDS Vaccine 2003 meeting established the initial proof of concept that centralized consensus HIV-1 genes can be both antigenic and immunogenic for wild-type epitopes.

Review of Presentations at the Vaccine 2003 Meeting. Korber (10) participated in a session describing several approaches to designing artificial sequences to attempt to better contend with the diversity of circulating forms of the virus. She discussed the derivation of the consensus and ancestral sequences, the balance of their theoretical limitations and virtues as vaccine antigens, and talked about how one could derive such sequences to represent either the HIV clades or the entire M group. The potential for mis-focusing of vaccine responses to epitopes that are no longer relevant in the circulating population by using an ancestor, was contrasted with the possibility of missing epitopes which frequently evade the immune response, but tend to revert back to a susceptible form upon transmission to a new host due to fitness constraints if one uses a modern consensus. The rationale for and design of the M group consensus/ancestor sequence called CON6, that is currently under testing for antigentic and immunogenic properties, was described. Korber encouraged the inclusion of these artificial antigens in polyvalent vaccine formulations, as well as supplementing them by more extensive cocktails that could potentially provide better coverage of highly immunogenic regions.

Haynes (14) and Weaver (9) discussed the initial characterization of the CON6 protein, and immunogenicity results. CON6 has promising characteristics with regard to retention of antigen features, proper folding, and stimulation of cross-reactive CTL. The CON6 protein binds many monoclonal antibodies that recognize important conformational or neutralizing epitopes, suggesting key antigenic properties are retained. The protein is weakly viable in a pseudotyping assay. The polyclonal sera from individuals infected with different clades of the virus bind to CON6 with the same intensity as within-clade proteins, at much more intense levels than typical between-clade responses. When used as an immunogen in BALB/c mice and guinea pigs, CON6 stimulated gamma interferon Elispot responses in T cells stimulated by peptide pools based on both B clade and C clade proteins. In contrast, a B clade immunogen gave rise to T cells that responded only to B clade peptides, and a C clade immunogen gave rise to T cells that responded only to peptides that were based on the C clade protein or

CON6, but not the B clade. These preliminary results suggest a potential for more extensive cross-reactivity and better converage between clades using a CON6 antigen rather than natural proteins.

Mullins (15) presented an interesting alternative perspective on deriving an ancestral sequence for use as a vaccine reagent. Differences in his strategies relative to those used by Korber include incorporating the hypervariable loops into the phylogenies to derive the ancestor – Korber excludes such regions from the derivation of the ancestor and re-inserts minimal hypervariable domains. Mullin's strategy results in an ancestral sequence enriched for N-linked glycosylation sites, relative to known isolates of HIV. His group is working on another approach called the COT sequence, or center of tree as this strategy might be less subject to the influence of outlier sequences than a conventional ancestor. His laboratory generated an ancestral B sequence that produced functional proteins with an R5 phenotype, and was able to elicit antibodies in a rabbit that could neutralize some primary isolates. Mullins also discussed experimental evidence from longitudinal studies in patients suggesting that soon after infection, patients tend to evolve back towards the ancestral strain, and as the infection progresses, diverge further away from it.

Mallal (16) and John (17) discussed yet another approach to designing antigens that might maximize the stimulation of cross-reactive, relevant responses. They have been able to track the influence of HLA presentation of CTL epitopes, immune escape, and fitness-imposed reversion to susceptible forms through the HIV positive local population in Perth. A regression analysis of variants relative to the population consensus, taking into account the HLA types of the infected host, revealed statistical support for both escape and reversion. On this foundation, they suggest an alternative strategy for designing artificial antigens. Rather than simply using the consensus, the most common amino acid in each position, they suggest using the second most common amino acid in highly selected positions. Thus in many cases they might be more likely to choose an amino acid that represents the susceptible form, if escape mutations are the most common form among the sampled variants. Indeed there comparison to the database of known epitopes suggests this strategy may confer an advantage and reconstructs additional possible CTL epitopes relative to other strategies.

Several other presentations had bearing on how one might think of immunological cross-reactivity among variants. Although the majority of global HIV-1 infections are acquired through heterosexual contact, this mode of infection is inefficient and generally involves transmission of only a subset of viruses from amongst a complex quasispecies. Whether this bottleneck in transmission occurs by chance or through selection of a particular biological property remains unresolved, yet may be of importance for vaccine design.

Hunter (13) presented a study demonstrating that among 8 donor-recipient heterosexual couples from Zambia, sequences that were representative of smaller and less glycosylated forms in the donors tended to be the only forms detected in the recipients soon after they became infected. This indicates either a fitness advantage at the point of transmission, or the rapid outgrowth of a variant with short hypervariable loops that are less glycosylated. Furthermore, the transmitted, expanded variants were all highly susceptible to neutralizing antibodies in the donor's concurrent sera, while the

donor retained a spectrum of neutralization sensitivity among PBMC and plasma variants sampled. Finally, pooled C subtype sera had a somewhat enhanced ability to neutralize the donors' variants, but again the forms sampled in the recipients were uniformly highly neutralization sensitive.

Polyvalent Vaccine Approach To Overcome HIV-1 Diversity. Several groups presented initial results at AIDS Vaccine 2003 using polyvalent formulations that are designed to help contend with diversity. The vaccine research Center (VRC) is exploring the potential of a DNA vaccine that carries the gag, pol, and nef proteins from a clade B isolate, and an env from a each of clades A, B, and C. Graham (50) presented encouraging levels of CD4 and CD8 T cell responses to peptide pools.that corresponded to the vaccine genes, with the greatest level of response to Env directed against the A immunogen. The greatest response being directed against Env may reflect a difference between vaccine responses and natural infections. Flow cytometric intracellular cytokine staining for IFN and IL2 was more sensitive than gamma-IFN Elispot, as both IL2 and IFN were measured outcomes.

Slobod (19) presented an approach with a 23-valent envelope vaccine (polyEnv1), including variants from several clades, as well as variants with different neutralization resistance profiles and monoclonal antibody binding profiles. They have developed a prime, boost, boost strategy involving a DNA prime, vaccinia boost, followed by a protein boost. They determined it is the superior combination strategy in animal studies using rabbits and mice. Currently they are testing each component separately in phase I human trials. Another interesting start on a polyvalent vaccine was presented by Lu (20) using a DNA prime, protein boost strategy. Rabbits were immunized using either monovalent, 3-valent, or 8-valent vaccines with HIV envelopes derived from multiple clades. Immunization-induced antibody reactivity was tested against 18 primary isolates, again from multiple clades. Multivalent vaccines elicited high titer neutralizing responses that were more broadly neutralizing than the monovalent vaccine. The 3-valent vaccine gave the most breadth and highest titer.

Ma (21) presented data demonstrating that, while an exhaustive analysis of immunogenicity of gp120 V3 motifs for induction of antibodies that broadly neutralize HIV-1 primary isolates, the most broad antibodies that could be induced by the most immunogenic V3 motifs (that all clustered around the subtype B consensus V3 sequences) were similarly limited in breadth to that described for the best human anti-V3 neutralizing antibody, 447-52D (J. Binley, personal communication, see below). In addition, all V3 motifs that induced neutralizing antibodies, induced responses that neutralized the same isolates, which were the traditionally more easy to neutralize isolates. No V3 motifs were found that induced antibodies that neutralized broadly outside of subtype B. Liao (22) reported BaL gp120 induced antibodies that neutralized ~ 40% of primary HIV-1 isolates, and Kim (23) reported similar results with ADA gp140 trimers. Much of the neutralizing activity of anti-gp120 sera are due to V3 reactivity, and it will be important to determine the species of antibodies induced by trimers. Thus, much basic research needs to be done to optimize any type of practical polyvalent approach to HIV-1 immunogen design. (See neutralizing antibody section below).

Finally, the VaxGen trials have ended in both the US and Thailand, and no efficacy was shown in these trials. It will be important to determine if the reactivity of the best neutralizing antisera reported at the AIDS Vaccine 2003 meeting has any additional breadth than the human sera from the failed VaxGen trials.

Recommendations For Design of Strategies to Overcome HIV-1 Diversity

1) Formalize the development of a database of newly transmitted HIV-1 full-length sequences for construction of subtype and group centralized sequences for antigenicity and immunogenicity studies. Include the sequences of breakthrough isolates from all HIV-1 vaccine trials.

2) Make the sera available from all clinical trials so that comparative studies can be performed between sera generated against all newer immunogens to determine if any induced antisera have more breadth than that generated by immunizing with AidsVax immunogens.

III. Development of Immunogens and Animal Models to Optimize Induction of Durable Anti-HIV-1 T and B Cell Responses

Overview of CTL-Induction Vectors. Considerable momentum continues to gather in moving CTL-based vaccines for HIV into clinical trials. However, there are potential "Achilles' heels" for each of the approaches currently under development. The level of immunogenicity of DNA vaccines in humans remains disappointing. Novel strategies for enhancing plasmid DNA vaccine immunogenicity with adjuvants are therefore needed. The most promising of the live recombinant vectors in monkey studies, recombinant adenovirus serotype 5, is likely to be less immunogenic in humans than hoped because of pre-existing immunity to the adenovirus serotype 5 vector (24). Therefore, the studies to generate vectors from unusual human adenovirus serotypes (i.e., ad35, ad11, etc.), chimpanzee adenoviruses, and novel chimeric human adenoviruses bear careful consideration. Adeno-associated virus (AAV) (25), although providing durable anti-HIV-1 immune responses, may also be disappointing as a vaccine vector in humans because of pre-existing anti-vector immunity, vesicular stomatitis virus (VSV) may prove too neurotoxic for human use, and production hurdles for some of the recombinant alpha virus vaccines may prove insurmountable. Considerable basic work in these areas is therefore needed to create a CTL-inducing vaccine modality that is both safe and highly immunogenic in humans.

Review of Relevant Abstracts From AIDS Vaccine 2003 Meeting. Regarding pathogenesis and adjuvants, there were essentially no abstracts that described incremental progress or new opportunities towards a better vaccine. Benchley (26) showed continuing insights into the T lymphocyte subsets containing HIV DNA with the implications regarding lymphocyte dynamics and HIV susceptibility.

An area of research critical to HIV-1 vaccine development regarding durability of responses, was highlighted in talks on characterization and optimization of induction of memory T lymphocytes by Ahmed (27) and memory B cells by Lanzavecchia (28).

Ahmed argued that upon antigen exposure a subset of proliferating T lymphocytes is programmed to become either effectors or memory cells. The latter can be identified by the expression of high levels of IL-7 receptor using labeled IL-7 receptor-alpha MAb. These cells undergo homeostatic proliferation and maintenance of numbers. IL-7 deficient mice are non-permissive for memory cell survival and IL-15 KOs are permissive.

Lanzavecchia argued, using tetanus toxoid and vaccinia antibodies, that maintenance for decades of steady state levels of antibody in humans without antigen boosting is directly correlated with the numbers of antigen specific memory B cells originally induced by immunization.

These two sets of observations provide specific targets for efforts to develop adjuvants and immunization strategies with the objective being the optimization of the induction of such memory B and T cells to HIV antigens. Also optimizing the generation of these memory T or B cells and specifically quantifying them during the evaluation of a vaccine strategy possibly will be a potentially useful objective for measuring long term protection in contrast to measuring the number of effector T cells or peak antibody levels acutely after immunization.

Finally, Rudensky (29) described the potential for modulating T regulatory cell function, and their regulatory gene, FOXP3, for modulating vaccine responses.

Recommendations For Development of Immunogens and Animal Models to Optimize Induction of Durable Anti-HIV-1 T and B Cell Responses

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7. Develop new animal challenge models to determine protective anti-HIV-1 T and B cell responses. These models should include low dose mucosal SHIV (to test Ab-based vaccines) and SIV challenge models.

8. Test mixtures of vectors for optimizing HIV-1 vaccine immunogenicity.

9. Consider a new effort to define the correlates of protection for *nef*-deleted attenuated SIV, as these correlates are still ill-defined. Define the role for other HIV regulatory genes as vaccine components.

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11. Define correlates of protection to HIV in other animal models of SIV/SHIV infection and in human clinical trials.

12. Define the comparative roles of free virus and cell-associated virus in the pathogenesis of HIV-1 infection, i.e. for design of effective vaccines, we need to know more about transmission modes at mucosal and systemic sites.

13. Encourage development of a "Human Challenge Model" of HIV-1, wherein HIV-1+ patients on HAART would be vaccinated with the more promising experimental immunogens. If they then elect to stop receiving therapy, the ability of the vaccine-induced immune responses to prevent, delay or modify the subsequent increase in plasma viremia could be a useful way to gauge vaccine potency. However, it must be noted that this experimental system is likely to be a more stringent test of the potency of a vaccine, because the amount of replicating virus to be countered is greater than is involved in *de novo* infection, and because of the possibility that the pre-existing HIV-1 infection has already caused a significant level of immune impairment in the volunteers, despite their receipt of therapy. A mechanism would need to be found to provide the drugs for such a trial.

IV. Strategies For Design of Immunogens to Induce Antibodies that Broadly Neutralize HIV-1 Primarily Isolates

Overview. A general theme from talks at the meeting and from submitted or recently published work is that some legitimate progress is being widely made within the field. The ability of immunogens to induce primary virus-neutralizing antibodies is definitely improving. However, as noted below, at least some of claims of "success" are, in fact, based on the use of more favorable assay conditions for neutralizing antibody (Nab) quantification, rather than representing true progress in the design of better immunogens. It is also fair to say that no group has come anywhere close to inducing the levels of neutralizing antibodies (Nabs) that are required for sterile protection in vivo. Passive protection studies with MAbs and HIV-1+ sera in the SHIV-infected macaque model have, over the past three years, given us a very good idea of what levels of NAbs must be induced (work from Burton, Martin, Mascola, Moore, Ruprecht and others). Lower levels of NAbs may still be useful, however, and there remains uncertainty about what level of neutralization could be biologically significant. Nonetheless, there is some progress.

Neutralization Assays. Over the past year, several new assays for the measurement of NAb activity have become more widely used than they previously were. In general, these are based on the use of co-receptor-expressing transformed cell lines, with embedded or virus-containing reporter genes (luciferase etc). Often, Env-pseudotyped viruses rather than naturally infectious isolates are used. General experience, with important confirmation from the comparative study recently organized by David Montefiori, is that these assays are usually reliable. It is important that it be understood that the various new assays do NOT, in general, provide substantial increases in sensitivity over the traditional PBMC-based assay using natural isolates; their greatest benefit is an increase in simplicity and precision combined, in some cases, with greater throughput and economy. With some assays, there can be a modest sensitivity gain (perhaps 5-fold) which is useful, but not a decisive factor. There are some unresolved issues concerning some Env-pseudotype assays, but overall these concerns are probably minor.

The increased precision of the newer assays does, however, allow a modest degree of neutralization to be quantified and "statistical significance" to be declared when making comparisons. It is also the case that certain isolates and Env-pseudotypes, even from bona fide primary isolates, are atypically easy to neutralize. The use of neutralization-sensitive viruses in assays with a modest sensitivity increase, and the ability to accurately quantify minor increases in neutralization, have allowed some investigators to make the claim that "we can neutralize primary isolates" with sera raised against their own test antigen. Being able to neutralize selected primary isolates is certainly necessary, but it is not sufficient. As always, judgement has to be applied when interpreting the true meaning of some claims. In particular, statistically significant and biologically significant progress are not necessarily the same thing, but they are all too often equated with one another. Since it is quite possible for essentially the same data set to be interpreted differently by different investigators, there is an urgent need for standardization of assay systems (see above). David Montefiori's work and recent efforts from the IAVI Neutralizing Antibody Consortium (NAC) are both relevant here. Thus, it should be entirely possible for standard panels of test isolates, *env* genes and

assay protocols to be assembled and distributed to researchers who are actively making new, Env-based immunogens for NAb induction.

Env-CD4 Complexes. At least four groups have studied complexes of Env with CD4 or a CD4-mimetic (Institute for Human Virology [IHV]; Chiron; Merck; Weill Medical College; PHRI). It is generally accepted that the gp120-CD4 complexes induce reasonable titers of relatively broadly neutralizing antibodies. There remains, however, considerable controversy about whether the neutralization is achieved via anti-CD4 antibodies, which are always induced and so are present in the assays (unless specifically depleted). The IHV and Chiron groups insist that anti-Env antibodies are responsible; work presented from Merck at Keystone, and reputed to have since been extended, suggests otherwise. Most of the more knowledgeable "outsiders" remain concerned about the presence of anti-CD4; the general feeling is that the case for anti-Env as opposed to anti-CD4 has not yet been satisfactorily made. At the AIDS Vaccine 2003 meeting, Liao (22) reported that mab A32-constrained BaL and 89.6 gp120s stably expressed the CCR5 binding site and the CD4 binding site, but this strategy did not induce broadly reactive neutralizing antibodies. Moreover, a recent publication from A. Pinter's group in *Vaccine* using the humanized "XenoMouse" model reports that single-chain gp120-CD4 complexes fail to induce neutralizing activity in the test mice. In those mice, the human CD4 component is non-immunogenic, and no anti-CD4 Abs were in fact induced (30). The implication is, therefore, that the anti-CD4 Abs raised in other experimental systems may be directed against CD4, and not Env. Of course from one perspective it does not much matter what is responsible for neutralization per se, but the presence of anti-CD4 Abs is perceived, rightly or wrongly, to be a problem for human trials because of issues related to autoimmunity.

There is too little publicly available information yet available on CD4-mimetics to judge whether this possible solution to the anti-CD4 problem is going to work. The approach is scientifically sensible; whether it will be successful may become clearer over the coming year.

Oligomeric Env. Many groups are pursuing this concept, in one guise or another (too many to list by name here). Some claims of success are being made (although the above caveats need always to be borne in mind). Most groups are using uncleaved forms of gp140, based on various different Env sequences. It is clear that cleavage (or the lack of it) does affect the antigenic structure and processing of Env (published and unpublished work from Sodroski, Moore). Whether these differences matter from the perspective of immunogen design is not yet clear; cleavage could make matters better or worse.

Not all Env oligomers are "native" trimers; gp140s from some strains are prone to forming dimers, trimers and tetramers, the dimers and tetramers being aberrant forms. "oligomeric" should not be equated to "native" or "trimeric", but often is, particularly by groups that do not actually work on these proteins. Again, it is too early to tell whether the extent of oligomerization matters from the immunogenicity perspective; it remains possible that "aberrant forms" may be useful immunogens, or the converse.

R. Wyatt's group at the NIH Vaccine Research Center established a paradigm for the methodology that should be followed when evaluating the performance of Env-based vaccines. The need for controls is critical: these include the use of pre-bleed sera and hyperimmune sera to irrelevant antigens to establish assay baselines; the need to perform comparative immunizations with monomeric gp120 to gauge the relative immunogenicity of oligomeric gp140's; the importance of purifying IgG fractions from sera to avoid the possible complications caused by non-Ig components, including cytotoxic factors; the importance of immunodepletion protocols to remove V3 loop antibodies and thereby determine whether the immunogen has truly induced cross-neutralizing antibodies; the need to identify against what epitopes any NAbs have, in fact, been induced. These types of studies should become the accepted norm within the field - together with the use of more standardized assays and test viruses (see above), such practices would greatly facilitate comparisons between the results of different groups evaluating different concepts. The application of standard reference sera (e.g., relatively broadly neutralizing sera from HIV-I-infected humans, such as FDA-2, or from macaques involved in passive antibody-protection studies) would be helpful.

Wyatt's studies clearly show that a trimeric gp140 is superior to a monomeric gp120 based on the same YU2 sequence in terms of its ability to induce NAbs. Efforts to improve the performance of the gp140 are ongoing, as are the investigation of the potential of particulate forms of YU2 Env (liposome-based).

Fusion-intermediate Immunogens. Despite prominent setbacks in recent years, the overall concept of the fusion-intermediate immunogen remains valid, for reasons reviewed by Weiss (31) in her presentation. The fusion process is a conserved event, and the Env regions involved are among the most conserved segments of the complex. Antibodies can be raised against several gp41 regions that are involved in fusion, but they lack neutralizing activity under conventional conditions. The major constraint to their efficacy are time and space – can the antibodies gain access to their epitopes within the limited space available between the attached virus and the cell surface, and sufficiently quickly to trap the fusion-intermediate configurations before they disappear as the Env complex shifts it shape yet again? These are tough problems to solve. On a related point, it is now clear from a recent paper from Burton and Sodroski (32) why the X5 Fab to a CD4-induced epitope is potently neutralizing when the IgG version of the same antibody is not (indeed, this is true of all tested Fabs and IgG's to the CD4-induced epitope cluster). The Fab is sufficiently small to gain access to its epitope on gp120 after gp120 has attached to CD4, but the IgG molecule is too large to fit into the available space. Unfortunately, the immune system responds to antigens by making IgG's, not Fabs.

Consensus Env Sequences. The rationale for use of consensus sequences for Nab immunogens is that this could provide at least a partial solution to the problem of HIV-1 sequence diversity. It is now clear that Env proteins that are properly folded and which retain some functional activity can in fact be expressed (which was certainly not a given when the projects were initiated) (14). They appear to be performing in early stage immunogenicity studies at least as well as broadly comparable Env proteins based on more natural sequences. It should be noted that the variable loops (V1-V2,

V3) of at least some of the consensus Env proteins are not, in fact, consensus sequences, but are derived from an individual, natural isolate. Hence Abs raised to these variable regions will have the usual restricted ability to cross-neutralize. Nonetheless, the scientific solidity of the overall concept, and the magnitude of the sequence diversity problem, renders these projects important. (See discussion above regarding the centralized gene approach).

On a related topic, there is no consensus among investigators as to what Env sequence to select as the basis of immunogen design. The decisions taken are generally based on rational, defensible principles, but could easily turn out to be mistakes. Emerging information suggests that it is not necessary to choose wild-type HIV-1 genes from the country where a vaccine is to be tested. It is important that additional knowledge be gained in this area, to better guide the decisions that have long-term and expensive consequences. The IAVI NAC group is attempting to address this critical issue; the NIH should do likewise, preferably in coordination.

Broadly Neutralizing Mabs. The IAVI NAC group has recently completed an extensive re-analysis of the ability of NAbs to cross-neutralize primary isolates from different genetic subtypes, using an Env-pseudotype assay at Virologics, Inc (J. Binley et al, personal communication). The results are very similar to what was published by several groups in the mid-1990's, and to the outcome of the Antibody Serology Project study organized by Pat D'Souza at that time. Hence the results provide a useful validation of the use of Env-pseudotype assays for neutralizing antibody detection. The NAC would no doubt be prepared to share the results with NIAID, upon request (Dennis Burton or Wayne Koff). One reason for such coordination is the importance of establishing consensus virus test panels and methodology for NAb quantification to allow comparisons to be readily made between work in different laboratories (see above).

The results of the NAC study re-affirm that the *bona fide* broadly neutralizing MAbs are b12, 2G12, 2F5 and 4E10. The 4E10 MAb has unusually broad cross-subtype activity, albeit at a modestly reduced potency compared to b12, 2G12 and 2F5. The IgG form of the X5 Fab almost completely lacked the ability to neutralize primary isolates. Two V3 MAbs were also tested: 447D (Zolla-Pazner) and 58.2 (Repligen). Neither performed well, with 447D being slightly the better of the two. Almost all of the "hits" by the V3 MAbs were within subtype B, with very few against viruses from other subtypes. When the V3 MAbs did "hit", however, they usually did so with significant potency. These results confirm what has long been known: the V3 loop is not a well conserved target for NAbs, although there are some conserved features within a subtype (or at least within subtype B). The 447D antibody should not described as a "broadly neutralizing MAb" akin to b12, 2G12 and 2F5: it is not, for its activities are very much more sequence restricted.

As discussed by Wilson (33), the best neutralizing antibodies all have highly unusual features which is potentially quite disturbing from the perspective of being able to induce antibodies of similar specificities by vaccination. The 2G12 MAb is truly bizarre, unprecedented in the annals of MAb structures; the b12 and 2F5 MAbs have unexpectedly long CDR3 loops (although this may be more true of human rather than mouse MAbs than has been appreciated); some MAbs to CD4i epitopes are sulfated to

mimic the N-terminal domain of CCR5 (work of Sodroski and Robinson); the recognition by 447-D of main-chain atoms more so than ones from the amino-acid side chains. The rarity of the broadly neutralizing MAbs is a reflection of the difficulties the human immune system has in overcoming the defenses erected by HIV-1 upon its Env glycoproteins.

HIV Env Variable Loops. There is an ongoing attempt to resurrect the V3 loop as a bona fide vaccine target. However, the data for focusing on the V3 loop are not strong. First, the evidence for cross-neutralization by V3 MAbs is limited (see above) despite claims to the contrary. Second, the notion that the V3 loops of R5 and X4 viruses have structural homology to a surface loop (the "40s loop") on CC- and CXC-chemokines respectively (34) represents a substantial over-interpretation of structural data. Third, "40s loop" is not, in fact, a component of the binding site on chemokines for CCR5 or CXCR4. Much of the "40s loop" is not even surface accessible, but is actually buried in the hydrophobic core of the chemokine molecule. The most solvent-accessible Arg and Lys residues on the "40s loop" of RANTES, for example, can be mutated to Ala without affecting CCR5 binding. Most studies implicate the "40s loop" as actually being involved in chemokine binding to cell surface glycosoaminoglycans, and not binding to GPCRs like CCR5 and CXCR4.

Attempts to use the V3 loop as a vaccine antigen appear to be limited by the sequence diversity problem and the poor exposure of the V3 loop for neutralizing antibodies on a substantial number of HIV-1 primary isolates. As noted above, it is common practice by most groups to try to immuno-deplete V3 Abs from sera to try to gauge the ability of the test immunogen to induce broadly neutralizing NAbs, without interference from isolate-specific antibodies of this variable region. Ma (21) presented evidence at the AIDS Vaccine 2003 meeting that, even after an exhaustive immunogenicity screen of over 60 different V3 motifs predicted to have conserved higher order structures, broadly neutralizing antibodies could not be elicited against primary isolates. The induced anti-V3 antibodies were predicted to have no greater breadth than antibodies to some of the most immunogenic gp120s, again demonstrating the limited utility of the V3 loop as a practical neutralizing antibody target. Most of the traditionally-more-difficult-to-neutralize HIV-1 primary isolates were resistant to the most broadly reactive anti-V3 responses. Thus, if the V3 loop is to have any utility in the future as a component of an immunogen, it will have to be in concert with immunogens that induce a specificity of antibody able to up-regulate the expression of conserved V3 motifs on difficult-to-neutralize HIV-1 primary isolates. Whether this kind of theoretical synergism can be accomplished without "diverting" the immune response from conserved epitopes is doubtful.

Pinter (35) showed that two variants of HIV, one highly sensitive to neutralizing many antibodies (SF162), one highly resistant (JRFL), could have their neutralization susceptibility reversed by exchange of the V1V2 domains. The change in neutralization sensitivity pertained to many monoclonal antibodies that have epitopes that bind with high affinity to both envelopes, and seems to be independent of epitope location. This study is interesting to consider in the context of Derdeyn's finding (13, discussed above) that neutralization sensitive variants are all that was sampled soon after transmission, and that these variants tend to carry short V1V2 loops. Although Pinter provided

evidence that the V1/V2 loop remains an important determinant of neutralization sensitivity (35), the same concerns are present for V1, V2 loops as for V3, in that the diversity of subtypes and quasispecies make the variable loops unattractive targets for immunogen design.

Johnson (36) described factors affecting neutralization sensitivity of SIVmac239 (V1/V2 deletion; glycosylation sites deletions; cytoplasmic tail truncation). At least some of these mutations have diminished envelope incorporation into virions accounting for greater neutralization sensitivity.

Epitope sShielding. Several groups (Burton, Wyatt, Sattentau and no doubt others) are trying to focus the immune response towards the induction of neutralizing, and not non-neutralizing, antibodies, by masking non-neutralizing epitopes on the candidate immunogen. This concept is perfectly sensible, and it is clear that non-neutralizing epitopes can be masked by, for example, structure-based, rational mutagenesis. It is too early to tell whether the elimination of the unwanted epitopes does, in fact, focus the B-cell response on the more desirable regions of Env; again, time will tell.

Env presentation. Research on adjuvants and other methods to better present Env to the immune system has long been neglected compared to other areas of Env biology. The use of particulate forms of Env (Wyatt and others) is one approach worth pursuing, as are inactivated virions expressing native Env (Lifson, Arthur and others). A third important approach is to use dendritic cells to present Env-antigens (e.g., Bhardwaj). Cytokine adjuvants are being studied by some groups, but little real progress appears to have been made recently. Of the more conventional adjuvants, several are being used by different groups in different experimental systems, again complicating comparisons between different data sets. Some standardization would be useful here as well.

New Data on Structure of HIV Envelope from the AIDS Vaccine 2003 Meeting. Wyatt (37) discussed efforts to study the envelope trimer. Why is gp120 a poor immunogen? Flexibility, immunodominance of variable epitopes, and a heavy coating of glycans all seem to contribute. He believes that the env *trimer* probably drives the rare broadly neutralizing antibodies. They have made fusion proteins of the gp160 ectodomain (cleavage deficient) with trimerization elements: GCN4, fibritin, and there are on-going studies to determine whether any of these might elicit more broadly neutralizing antibodies than gp120. They use proteoliposomes to display the trimeric immunogen. There is controversy as to whether there are significant structural differences between cleaved and uncleaved. (N.B.: there are very minor changes when flu HA0 is cleaved to HA1/HA2).

Roux (38) showed results of EM tomography of SIV (both negatively stained and cryo preparations). They have used a mutant (in the gp160 cytoplasmic tail) that puts 70-80 gp160 trimers on each particle. A trimer looks like a 3-blade propeller on a stalk. They have fit Peter Kwong's trimer model to the 3-blade propeller, with reasonable success. (Note that even if that fit turns out to be right, however, we still do not know very much about the prefusion conformation of gp41 or the contacts between gp41 and gp120.)

Weiss (31) reviewed the gp41 fusion transition. There are broadly neutralizing Abs that bind near the "base" of gp41 (proximal to the TM segment). Do these Abs neutralize by preventing the conformational change, interfering with close contact of the two membranes, or by interfering with higher-order interactions (inter-trimer)? The binding of 2F5 goes down after the fusion transition is triggered, whereas that of D10 (a related Ab studied by P. Earl) goes up. Weiss showed that if you trap a fusion intermediate with C peptide (outer layer), you still get binding of 2F5. It does not, however, prevent progression to the 6-helix bundle. Why can't you generate neutralizing antibodies to the fusion intermediate? (N- and C- peptides of gp41 are immunogenic but produce no neutralizing or fusion-inhibiting activity.) It is ot clear whether the problem is steric or kinetic. Regarding the structure of the broadly neutralizing 4E10 Fab in complex with a peptide containing the sequence of the 4E10 epitope (near "base" of gp41, just before it enters membrane). Could this structure aid in the design of high-affinity mimetopes to use as immunogens for eliciting 4E10-like neutralizing responses? (33, 39).

Schulke and the Weil Medical College/Progenics Team (40) are studying a gp140 trimer in which the gp120-gp41 association is stabilized by a disulfide bridge and the gp41 conformational change is inhibited (so they intend) by an Ile to Pro mutation (presumably to prevent helix formation in a segment they believe to be non-helical in the trimer but known to be helical in the post-fusion state). Stable trimers of this protein (they call it SOSIP gp140) can be isolated. The protein is recognized by neutralizing, but not by non-neutralizing Abs. Immunogenicity studies in small animals have begun.

Chiron scientists reported a trimeric gp140, derived from SF162 by partial deletion of V2 and it seems to be a stable timer (41). In a DNA-prime, protein-boost study in rhesus macaques, some neutralizing antibodies against heterologous subtype B primary isolates were detected, as well as protection upon challenge with pathogenic SHIVSF162.

Thus, our understanding of the trimer structure of gp160 Env has advanced a bit but remains fuzzy. The trimers now reported for HIV gp140 are more stable than ones described previously – a solid biochemical advance. It is important to note that SIV gp140 gives much more stable trimers, without the need for trimerization tags, disulfides, etc.

Recommendations For Strategies For Design of Immunogens to Induce Antibodies that Broadly Neutralize HIV-1 Primarily Isolates

1. Increase funding of basic research to develop novel approaches for immunogen development for neutralizing antibody induction. Ensure continued and robust funding of Innovations grants programs, HIV-RAD and IPCAVD programs. Define how we rank order the need for basic research, and evaluate funded programs.

2. A bridge program or process is needed for successful R21 AIDS Vaccine Innovative grants to move their products forward, such as an R33 program.

3. Develop a database of newly transmitted HIV isolates (see #1 above under strategies to overcome HIV-1 diversity).

4. Develop a standard panel of isolates to compare immunogens in an organized way to rank-order their potency for induction of neutralizing antibodies. Small but real improvements can be significant, pointing the way to viable strategies and possible combinations of strategies that would lead to greater potency. (This has already been planned by John Mascola and David Montefiori). A workshop aimed at gathering suggestions for a standard panel of HIV-1 primary isolates sponsored by the DAIDS, the HVTN and the AVRWG was held on Jan. 6, 2004. Another goal of the workshop was to discuss a level of cross-neutralizing activity that warrants advancement to phase II and III trials.

V. Clinical and International Research Issues

Critical events this year included the reports of the US VaxGen Trial 004 and the Thai VaxGen AIDSVAX B/E as showing no efficacy, and the beginning of RV144 Phase III trial in 16,000 subjects using the AIDSVAX B/E bivalent gp120 mixture as a boost for ALVAC vCP1521. This ALVAC contains the gp120 of a subtype E, gp41, gag and protease of LAI (subtype B).

Presentations from the AIDS Vaccine 2003 meeting that were of interest included the following abstracts. Mast (42) reported pre-existing Ad5. Antibodies may impair the ability of Ad5 vectors to elicit desired immune responses, so the investigators evaluated the Ad5 seroprevalence at 6 international sites. Prevalence of Ad5 antibodies was 91.2% at five international sites (Brazil. Cameroon, Malawi, South Africa, and Thailand), and 61.2% in the United States. These data will likely have implications for the use of Ad5 as a vaccine vector (see discussion of vectors that induce CTL above in III).

In the symposium "HIV Incidence and Risk Behavior in International Cohort Studies", Coutinho (43) presented HIV incidence and HIV risk behavior in the Amsterdam Cohort Studies of HIV uninfected homosexual men (HM) and drug users (DU), who have been followed every 3-6 months since 1985. Among HM, UAI decreased initially in the 80's and then increased again in the 90's; both gonorrhea and syphilis rates decreased from the 80's to 90's, but have rebounded to 80's levels in 2001-2002, whereas HIV incidence rates decreased dramatically from the 80's to 90's and more recently have fluctuated between 0 and 2%. Among DUs, there were significant decreases in injecting and sharing behaviors following the 80's, paralleling a sharp decrease in HIV incidence from 7.5% in 86 to
0% in 2002.

Bartholow (44) presented data from the recently completed 36 month North American AIDSVAX B/B RCT among 5108 MSM and 209 high-risk women. Initial decreases in UAI and UVI were noted between the 0 and 6 months visits, with gradual increases, though not to baseline levels, by 36 months. The HIV incidence remained relatively constant during the trial between 2.5% and 3.4%.

Van Griensven (45) presented behavioral data from the recently completed Thai AIDSVAX B/E RCT among 2,545 intravenous drug users in Bangkok. Injection and sharing both decreased respectively from 94% and 33% at baseline to 56% and 16% at 36 months.

Hoffman (46) presented data from a cohort of 600 high risk women followed for 30 months in Mbeya, Tanzania. The initial HIV prevalence was 68%, and the follow-up rate at 30 months was 84%. 34/192 HIV-uninfected women seroconverted with an HIV incidence in the first year of 14% and in the second year of 4%. Consistent condom use with casual partners rose from 20% to 67% after 2 years, but remained low with permanent partners throughout follow-up Kempf (47) reported on "Predictors of Enrollment and Retention for HIV Discordant Couples in Zambia". He demonstrated among 1067 discordant couples with HIV+ women and 928 men discordant couples, 14% and 8% were ineligible, only 72% and 79% actually enrolled, and 30% and 25% were lost to follow-up, illustrating some of the challenging in recruiting and retaining this population.

McCutchan (48) spoke on "Building an HIV-1 full-genome sequence database". She found that genetic sequencing of HIV-1 is an important adjunct to vaccine trials as it provides a description of the viral strains that will challenge vaccine candidates. The US Military HIV Research Program reported on 266 full-length sequences from Cameroon, Ethiopia, Kenya, Tanzania, Thailand and Uganda demonstrating subtypes A, B, C, and D, as well as AE, AG, BE, CE and other recombinants.

Jack (49) reported on a multi-site phase II trial of ALVAC-HIV vCP1452 alone and combined with gp120. Volunteers were vaccinated at 0, 1, 3, and 6 months and followed for 18 months. 160 volunteers enrolled, follow-up to be completed in 2004. Retention rate at 9 months was 97%, and side effects have been minimal. This was an example of a successful completion of a phase II multinational trial.

Cox (50) compared immune responses to *env* and *gag* after ALVAC 205 (divergent envelope constructs in *env* - B/E, but same B gag) in 60 US and 180 Thai volunteers. CD8 restricted pCTL activity to *env* was found in only 14% of US and 13% of Thai volunteers, and to *gag* in only 16% of US and 7% of Thai volunteers.

In the "Workshop on International Site Development" (Abstracts 51-62), this preconference workshop included a series of presentations describing international site development by the Department of Defense, the HIV Vaccine Trials Network (HVTN), International AIDS Vaccine Initiative, the Rwanda/Zambia HIV Research Group, and, as well, descriptions of HIV trial site development in Thailand, Kenya, and Haiti. Two presentations were given describing the development of laboratory support for HIV vaccine trials in Thailand (55) and by the HVTN (56). Willingness to participate in HIV vaccine trials was assessed in Brazil (57) and found to be 60% among 1000 subjects, and in Argentina (59) and found to be 54% of 563 subjects.

The design of the vaccines reported in the abstracts above and in preparation for multiple clinical trials in both the US and at international sites raises a number of questions. First is the distribution of similar vaccine prototypes by subtype and by vector. A critical question to answer is whether it is rational to have a myriad of different or similar immunogens simultaneously being tested in the same vector, such as MVA. Similarly, the NIH is now supporting development of Tanzanian, Indian, South African, China and other subtype C immunogens. It is critical to perform proof of concept studies quickly to determine if site-specific immunogens are required, and to determine if centralized immunogens are superior to wild-type immunogens. If either site-specific immunogens are not required, or centralized immunogens are superior to wild-type immunogens, then the pipeline can be quickly focused on those immunogens with the

most utility. Similarly, focusing on specific vectors that are not hampered by preexisting immunity, poor immunogenicity, and brief duration of induced immune responses is critical (see section III above). Finally, the roles of the HVTN, PAVE, AVRWG and other groups in decision-making regarding movement of candidates through the translational pipeline needs to be clarified.

Recommendations For Clinical Trials And International Research Issues

1. Establish and further define the process and criteria for moving candidate HIV-1 vaccines into Phase I, II, and III clinical trials, to include vaccine candidate cost and manufacturability.

2. Coordinate the myriad of trials organizations, and continue the organizational efforts of PAVE by DAIDS with HVTN, CDC, DoD and other relevant parties, e.g. IAVI.

3. For preclinical and clinical development of vaccine products, develop the expertise for SOP development for data management, trial end-point validation, and clinical trial site monitoring, and to make these SOPs available to the vaccine development community.

4. DIADS should continue to pursue mechanism of research and development that do not rely on traditional peer-reviewed mechanisms, to be able to quickly fill research gaps and move products forward.

5. DAIDS should begin to target the HIV Team Contracts, HIV-RAD, IPCAVD, and R21 grants to problems that are needed to be addressed and away from concepts that are already being addressed in the NIH research portfolio.

6. Establish a centralized effort to compare serum and cellular reactivity induced by HIV immunogens prior to entry of immunogens into clinical trials. The important point is that all critical immune assays be unified under central standard operating procedures and possibly at a central laboratory in order for immunogenicity data to be compared. For example, continued support for the NIAID/DOD GLP QC and Immunogen Comparison Laboratory effort and consider expansion of capacity, in order for a central site for immunogen testing to be developed.

VI. Community Issues

There were several key community issues highlighted at the AIDS Vaccine 2003 meeting. Montoya (63) reported on U.S. subject's attitudes toward HIV vaccine research. Those surveyed said they were supportive of AIDS vaccine research, but they lack knowledge of the issues surrounding this research. In a discussion of the VAX

004 trial, Collins (64) noted that more women and people of color must be involved in, and recruited into, AIDS vaccine trials. Advance work is needed to ensure global access and appropriate distribution. Biotech companies need to be encouraged to be involved in AIDS vaccine research, but they need assistance and support in several areas. In a discussion led by Wakefield (65), community mobilization was emphasized to be necessary for trials to succeed, but it is labor intensive, costly and takes time. There are a variety of markers that can be used to measure community readiness for trials. Community mobilization work has begun, but in a limited number of locations around the world.

An important symposium was presented on "Intellectual Property" (66-70). A database developed by Morrison and Foerster showed that there are over 1000 patents that apply to AIDS vaccines (66). Many of these patents overlap. Depending on who holds these patents, the system can either advance private research or inhibit collaboration and exploration of new approaches. Three concepts were discussed that need further attention are: 1) patent pooling, 2) agreements not to sue in early stage research, and, 3) novel IP arrangements for developing world markets (66-70).

There were two poster sessions (sessions 64 and 65) on community approaches and policy and preparedness issues. A variety of community preparedness approaches and materials were presented. Several posters documented the connection between community education and engagement with AIDS vaccine trial recruitment. These included:

- A study in Ghana reported on the need for delivery of more information and education to the community in order to "remove stigmatization for effective co-operation in testing HIV vaccines (71)
- In a survey of Brazilians, researchers found that unwillingness to enroll in AIDS vaccine trials was often related to, "misunderstanding or lack of information that may be addressed by appropriate interventions" (72).
- Researchers in Nigeria found that "community education and involvement will facilitate community mobilization towards effective preparedness for vaccine trials in Nigeria" (73).

Recommendations For Responding to Community Issues of HIV Vaccine Development:

1. Actively promote the recruitment of women and people of color into future HIV-1 vaccine efficacy trials.

2. Develop practical solutions to barriers of HIV-1 vaccine availability and potential manufacturing capability, in particular for developing countries. Define who should do this and how the discussion could be formalized to create a credible process.

3. Build adequate community information and education dissemination in the ongoing plans for trial network development, to ensure that expectations of the community are realistic.

VII. Summary

The relentless progression of the HIV-1 epidemic world-wide in spite of the world's efforts, emphasizes more than ever the need to accelerate, intensify and coordinate the process of HIV vaccine development. The field of HIV vaccine research has labored for nearly 20 years in the quest for an AIDS vaccine. Much has been learned and although some progress toward development of a safe, effective and practical vaccine has been made, major problems remain to be overcome. It has become apparent that if, indeed, it is possible to make an effective HIV-1 vaccine, then the successful and timely development of an effective vaccine will require focusing existing resources on clinical trials of the most promising candidates, coupled with concerted efforts in basic research to understand the correlates of protective immunity, and to develop novel solutions to neutralizing antibody immunogen design, vector design, and T cell immunogen design to overcome HIV-1 diversity. By synergizing basic research and clinical research efforts and focusing on the critical proof of concept experiments, an iterative process can continue to move forward to bring promising experimental HIV immunogens into Phase III trials.

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